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Elimination of the Immunogenicity of Therapeutic Antibodies¹

Lisa K. Gilliland,* Louise A. Walsh,[†] Mark R. Frewin,* Matt P. Wise,* Masahide Tone,* Geoff Hale,* Dimitris Kioussis,[‡] and Herman Waldmann^{2*}

The immunogenicity of therapeutic Abs limits their long-term use. The processes of complementarity-determining region grafting, resurfacing, and hyperchimerization diminish mAb immunogenicity by reducing the number of foreign residues. However, this does not prevent anti-idiotypic and anti-allotypic responses following repeated administration of cell-binding Abs. Classical studies have demonstrated that monomeric human IgG is profoundly tolerogenic in a number of species. If cell-binding Abs could be converted into monomeric non-cell-binding tolerogens, then it should be possible to pretolerize patients to the therapeutic cell-binding form. We demonstrate that non-cell-binding minimal mutants of the anti-CD52 Ab CAMPATH-1H lose immunogenicity and can tolerize to the "wild-type" Ab in CD52-expressing transgenic mice. This finding could have utility in the long-term administration of therapeutic proteins to humans. *The Journal of Immunology*, 1999, 162: 3663–3671.

We have long noted that the idiotypic determinants of multivalent mAb targeted to Ags on the surface of hemopoietic cells, such as Thy-1, CD5, or CD8 or even against a neutrophil surface Ag, are exceptionally immunogenic in vivo (1). This "cell-binding effect" limits the long-term use of Ab in controlling chronic conditions such as autoimmune diseases. CD4 is a notable exception; mAb to CD4 can induce tolerance both to itself and to soluble proteins (1). Although mAb allow specific targeting of cell populations bearing defined Ag such as CD52 (CAMPATH-1) (2–6), humanized forms can still elicit strong anti-idiotypic and anti-allotypic responses against foreign residues in the complementarity-determining region (CDR)³ sequences (7–10). These responses can prevent any therapeutic benefit to the patient (10, 11). In a single-dose-escalation study to monitor the effect of CAMPATH-1H in patients suffering from rheumatoid arthritis, 63% of patients developed antiglobulin responses and minimal clinical benefit was observed (10).

We have sought a universal strategy to circumvent the immunogenicity of cell-binding mAb so that Ab-based therapeutics can be used to their full potential. We know that the antiglobulin response to cell-binding mAb is a CD4⁺ T cell-dependent process typical of any foreign protein (12). We assume that CD4⁺ T cells recognize Th peptides derived from processing of the mAb to initiate the production of antiglobulins. If these peptides could be presented for tolerance instead of for activation, the antiglobulin response may be avoided. We based our approach on high zone tolerance induced by soluble protein. Historically, high doses of

soluble monomeric xenogeneic human γ -globulin (HGG) could induce tolerance in mice to immunogenic challenge with the aggregated form (13, 14). Tolerance of the animals required only that the T cells be unresponsive, although B cells also were inactivated at high tolerogen doses (15, 16). We reasoned, therefore, that a high dose of monomeric non-cell-binding variant of a therapeutic mAb could tolerize to all foreign epitopes (T cell and B cell) of the "wild-type" cell-binding form.

Here we demonstrate that the immunogenicity of a well-characterized and widely used humanized therapeutic mAb (CAMPATH-1H) can be eliminated by converting it to a non-cell-binding molecule. By definition, a non-cell-binding Ab cannot bind to the cell surface and will remain monomeric because of its inability to form complexes with Ag. In contrast to cell-binding Ab, non-cell-binding Ab will not form immunogenic aggregates irrespective of Ag-processing-related interactions with APC or monocytes. Nor can they interact with the FcRI high affinity receptor for monomeric IgG, as this receptor is normally occupied in vivo with autologous IgG. The primary interaction of non-cell-binding Ab may be by default with FcRn, the IgG protection receptor (17, 18).

We made minimal mutations to preserve as many of the T cell and B cell epitopes as possible for tolerance induction. Four mutated variants were constructed, three of which carried a different single mutation (SM1, SM2, and SM3) in the H2 loop, and one of which carried a double mutation (DM) in the same region. Two of these mutants (SM3 and DM) did not bind CD52. While wild-type CAMPATH-1H Ab monomers were immunogenic in CD52-expressing transgenic (Tg) mice, non-cell-binding SM3 or DM did not elicit responses. Moreover, nearly all mice pretreated with the non-cell-binding mutants became tolerant to challenge with multiple doses of the wild-type CAMPATH-1H mAb.

There are now more than 20 humanized mAb in clinical trials (19), and several of these, including CAMPATH-1H, have been used extensively in varied applications (e.g., the treatment of malignancies or prevention of allograft rejection). In organ transplantation, these reagents are often administered along with cytotoxic drugs or steroids that nonspecifically suppress antiglobulin responses. However, this approach exposes a patient to the dangers of generalized immunosuppression. In contrast to organ transplantation and cancer therapy (in which the patient's immune system may be compromised by disease), autoimmunity is an area in

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³ Abbreviations used in this paper: CDR, complementarity-determining region; HGG, human γ -globulin; SM, single mutation; DM, double mutation; Tg, transgenic; GPI, glycosylphosphatidylinositol; HRPO, horseradish peroxidase.

which mAb treatment may be particularly limited by neutralizing anti-idiotypic responses. These patients are relatively immunocompetent, and the diseases may well benefit from repeated treatments with therapeutic mAb. We have documented anti-idiotypic responses following repeated administration of CAMPATH-1H in rheumatoid arthritis (7) and multiple sclerosis (P. Rebello, A. Coles, and A. Compston, unpublished results).

Whatever the source of human or humanized Ab, be they from phage display, xenotransplanted mice, or even human Ig Tg mice (reviewed in Refs. 19 and 20), there is no reason to doubt that these too would elicit anti-idiotypic responses in humans. The process of affinity maturation will inevitably generate Ab to which patients may not be naturally tolerant. Moreover, genetic polymorphisms in the Ig locus (21–23) predict that not every patient would have had the opportunity to acquire natural tolerance to every potential therapeutic human Ab. In the future, we hope the tolerance strategy we have developed will allow Ab to be used to their full potential in treating both acute and chronic human diseases, without the need for additional generalized immunosuppression. This approach may also lessen the need to modify therapeutic Ab or other therapeutic proteins to be completely “humanlike” before clinical application.

Materials and Methods

Production of wild-type CAMPATH-1H Fab and CDR-swapped Fab mutants

An expression plasmid of the CAMPATH-1H Fab was generated by the method of Clackson et al. (24). The V_H and V_L gene fragments were isolated from CAMPATH-1H light chain and heavy chain expression plasmids (25) by PCR. Ig CH1 and C κ domains, and a previously described linker fragment (24), were assembled together with the CAMPATH-1H genes by PCR. The entire DNA fragment was then transferred to the pHEN vector (Ref. 26; a gift from G. Winter, Cambridge Antibody Technology, Cambridge, U.K.) using flanking *SfiI* and *NotI* restriction sites (Fig. 1A). Four unique restriction sites found in the framework regions between the CDRs, and unique *SmaI* and *HindIII* restriction sites introduced by PCR, were used to create the CDR-swapped Fab mutants. DNA encoding each CAMPATH-1H CDR was swapped with DNA encoding the corresponding fragment from the human myeloma NEW heavy chain (27) or REI light chain (28) (Fig. 1B). The CAMPATH-1H Fab fragment and the CDR-swapped mutants were expressed in *Escherichia coli* HB2151 cells. Supernatants were isolated from bacteria by centrifugation and were filtered through 0.45- μ m membranes. PMSF was added (50 μ g/ml). Expressed Fab were detected by ELISA using microtiter plates coated with goat anti-human Fab (Sigma, St. Louis, MO) and biotinylated mouse 9E10 anti-c-myc Ab (a gift of G. Evan, Imperial Cancer Research Fund, London, U.K.) followed by ExtrAvidin peroxidase (Sigma). Fab fragments were quantified by ELISA and were used at equivalent protein concentrations.

Generation of a recombinant form of the CD52 Ag

Mouse IgG2a C-region sequence (amino acid residues 216–446, EU numbering) was expressed with an aglycosylated form of CD52 (Fig. 2A). The CD52 signal peptide sequence was amplified from CD52 cDNA (3) by PCR using the primers 5'-TTGTGACCTACCAAAATGAAGCGCTTC-3' and 5'-TTGTCTGAGAGTCCAGTTTGTAT-3'. The CD52 coding region was amplified from the same template DNA with the primers 5'-TTGGATCCTAAGGCTGAGACGTGTACAC-3' and 5'-AGCTTCTCCCGGACTCCGGGTAAAGGACAAGCCGACACCAAGCCAAACC-3'. The mouse IgG2a C region was amplified using the primers 5'-TTTACCCGAGTCCGGGAGAGT-3' and 5'-ATACAACTGGACTCTCAGGACAAGAGCCAGAGGGCCCAATCAAG-3' and cDNA from an IgG2a-expressing cell line (NHM60.4.1.5). The gene fragments were assembled by splice overlap-extension PCR (29) and inserted into the pHAPR-1-neo vector (30). Stable transfection of BHK21 C13-2P cells was conducted by electroporation. Although the CD52 immunofusion protein was designed as a glycosylphosphatidylinositol (GPI)-anchored molecule (Fig. 2B), a fraction of the expressed protein was shed into the culture supernatant. This facilitated its purification by protein A column chromatography (0.1 M glycine buffer (pH 3.0) elution and 0.1 M Tris-HCl (pH 8.0) neutralization, followed by dialysis against PBS).

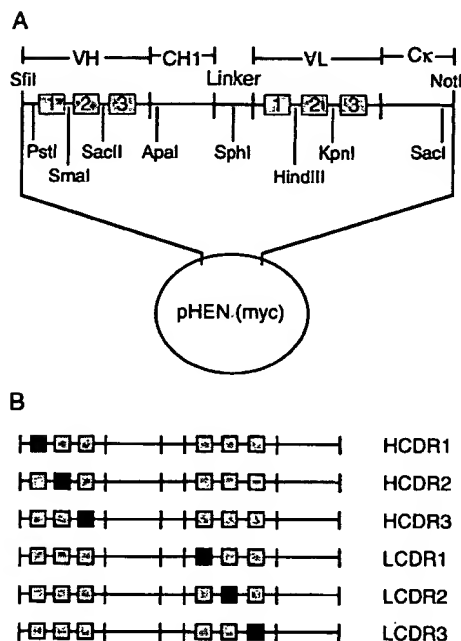


FIGURE 1. Generation of CAMPATH-1H Fab and CDR-swapped Fab fragments. *A*, Schematic diagram indicating relative positions of restriction sites in the CAMPATH-1H Fab fragment expression plasmids. *SmaI* and *HindIII* sites were introduced to allow swapping of individual CDRs (gray boxes). *B*, CDR sequences of CAMPATH-1H were replaced with corresponding CDR sequences of NEW (heavy chain) and REI (light chain), shown as black boxes, to generate six Fab mutants (HCDR1, -2, and -3 and LCDR1, -2, and -3).

Production of “minimal mutants” of CAMPATH-1H mAb and quantification of binding to CD52

Single or double mutations in the H2 loop of CAMPATH-1H mAb were introduced by PCR mutagenesis, using cDNA encoding the wild-type heavy chain of humanized CAMPATH-1H (25) as PCR template (Fig. 3). Three minimal mutants were generated that each had a single amino acid change in H2: SM1, LysH52b to Asp (K52bD); SM2, AspH52a to Lys (D52aK), and SM3, LysH53 to Asp (K53D). Additionally, one minimal mutant (DM) was created with two amino acid changes in H2: LysH52b and LysH53 to Asp (K52bD and K53D). CAMPATH-1H heavy chain DNA was amplified using a 5' primer that anneals to the leader sequence and an upstream *HindIII* site (25), and one of four 3' primers complementary to sequence encoding each of the mutations, generating 220-bp fragments. The DNA was also amplified using a 3' primer (complementary to sequence in CH1 flanking a unique *BstXI* site) and one of four 5' primers encoding each of the mutations to generate 440-bp fragments. The 220-bp and 440-bp fragments were then assembled by splice overlap-extension PCR (29) to generate four mutagenized DNA cassettes. A unique *PstI* restriction site located in V_H framework 1 (Fig. 1) and the unique *BstXI* site in DNA encoding human IgG1 was used to replace the wild-type *PstI*-*BstXI* fragment with each of the cassettes. Completed heavy chain DNAs were then transferred to a neomycin-selectable mammalian expression vector (pBAN-2), a derivative of pNH316 (32). CAMPATH-1H light chain cDNA (25) was introduced into pRDN-1, a variant of the pLD9 vector carrying a dihydrofolate reductase-selectable marker (32). Each heavy chain expression plasmid was cotransfected with the light chain construct using *N*-[1-(2,3-dioleoyloxy)propyl]-*N,N,N*-trimethylammonium methylsulfate (Boehringer Mannheim, Indianapolis, IN) into Chinese hamster ovary cells lacking endogenous dihydrofolate reductase activity.

Following selection and limiting dilution cloning, supernatants were screened for Ab by ELISA using microtiter plates coated with goat anti-human IgG (Amersham, Arlington Heights, IL) and detection with biotinylated goat anti-human κ light chain (Sigma), followed by ExtrAvidin peroxidase. To quantify binding to CD52, minimal mutants were captured in an ELISA using anti-human IgG-coated microtiter plates. Recombinant Ig-CD52 (5 μ g/ml) was added, followed by biotinylated anti-mouse IgG (Sigma) and ExtrAvidin peroxidase. Additionally, binding of the mutants

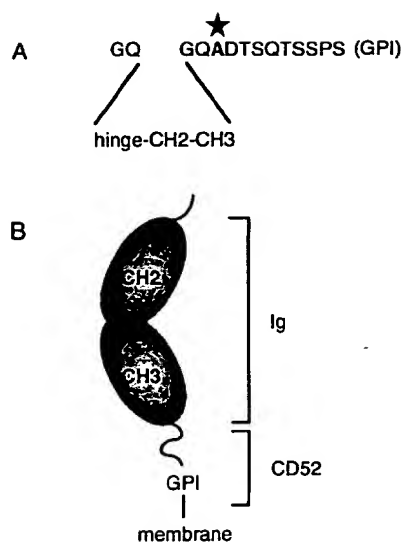


FIGURE 2. Production of Ig-CD52 fusion protein. *A*, Design of recombinant expression cassette. A portion of the mouse IgG2a C region (hinge-CH2-CH3, amino acids 226–478) was inserted downstream of the CD52 signal peptide and the first two amino acids encoding the mature protein of CD52 (–24 to +2). The entire CD52 coding region was then introduced (+1 to +37), with Asn3 changed to Ala (starred) to remove N-linked glycosylation. *B*, Putative structure of the Ig-CD52 fusion protein. The expressed protein comprises two amino acids (Gly-Gln) of the N-terminal region of CD52, the hinge-CH2-CH3 regions of mouse IgG2a, aglycosylated CD52, and the CD52 GPI anchor. Although illustrated as a monomer for simplicity, the hinge cysteines would allow dimerization of the fusion protein.

to CD52⁺ lymphocytes, isolated from the spleens of CD52 Tg mice, was detected by flow cytometry using biotinylated goat anti-human IgG (Amersham), followed by ExtrAvidin-FITC (Sigma). Cell staining was quantified using a FACScan II sorter (Becton Dickinson, Mountain View, CA).

Generation of Tg mice expressing CD52

To assess the tolerogenic potential of the minimal mutants in vivo, we generated Tg mice expressing human CD52 (CD52 Tg mice). A genomic DNA fragment containing the human CD52 gene (M. Tone, manuscript in preparation) was introduced into the genome of CBA mice by fertilized egg microinjection, as described (33). The 4.5-kb promoter region of human CD2 and the 3' locus control region sequences were used to direct high level, tissue-specific expression (34). Four CD52/CBA founders were established that transmitted the transgene. Peripheral blood staining of their

offspring using two-color staining and FACS showed that cells expressing murine CD3 also expressed human CD52. These mice were bred to homozygosity, and >95% of their T cells expressed high levels of cell-surface human CD52.

Preparation of deaggregated Ab

Each minimal mutant was purified from 10 liters of transfectant culture supernatant by protein A chromatography. Passage over a Superdex 200 gel filtration (size exclusion) column (Pharmacia Biotech, Piscataway, NJ) was then used to obtain monomeric material. Fractions containing monomer were dialyzed against PBS and sterilized by filtration (0.2 μM). The monomer was checked on an analytical size-exclusion column to confirm that the mAb did not dimerize following purification. CAMPATH-1H mAb purified in this way remains stable for many years unless subjected to aggregating procedures (data not shown).

Results

Defining CDRs used for Ag binding

To create a minimally mutated, non-cell-binding variant of CAMPATH-1H, we needed to determine which CDR residues were critical for interaction with Ag. Additionally, it would be useful to identify CDRs that did not play a role in Ag binding (if any). An Ab may not use all six of its different V-region CDRs, even for binding to large proteins (35). We worked to define a general strategy that could be applied to creating a nonbinding minimal mutant of any immunogenic Ab-based therapeutic protein.

To identify CDRs that were involved in CD52 binding, we created six CDR mutants of CAMPATH-1H as soluble, myc-tagged Fab fragments using a rapid *E. coli* expression system featuring the pHEN vector (24, 26, 36). Each Fab mutant contained one CDR derived from the V_L or V_H sequences that provided framework residues during CAMPATH-1H humanization (37). This strategy was adopted to maintain the basic structure of the CAMPATH-1H V-region domains, and thus of the supported CDR loops, in the CDR-swapped mutants. The Ig-CD52 immunofusion protein was then used to screen for the ability of the CDR-swapped mutants to bind Ag. Initially, methanol/chloroform-extracted Ag (3) was used to coat ELISA plates, but specific binding of the Fab fragments was difficult to measure reproducibly (data not shown). As an alternative, soluble bivalent Ig-CD52 immunofusion protein was generated to provide a source of Ag. Binding of Ig-CD52 was observed only with the wild-type CAMPATH-1H Fab fragment and with the LCDR1 and LCDR2 mutants containing RE1 CDR1 or CDR2 in the CAMPATH-1H light chain (Fig. 4). These results suggest that CDR1, -2, and -3 of the heavy chain and CDR3 of the light chain are necessary for recognition of, and binding to, the CD52 Ag. The findings were confirmed using single-chain Fv to control for possible variations in binding caused by unassociated heavy and light chains (L. Walsh, M. Frewin, and O. Dolezal, unpublished data).

Minimal mutations of CAMPATH-1H disrupt Ag binding

CDR swapping focused our attention on the three CDRs of the heavy chain and CDR3 of the light chain as regions for site-directed mutagenesis to create a non-cell-binding form of CAMPATH-1H. In total, 26 residues are found in these loop structures (6 in H1, 6 in H2, 8 in H3, and 6 in L3) (38). Two Ab variants carrying single-residue mutations were made based on structural predictions from molecular modeling. These studies suggested that a charge difference of K52b to D (SM1), or D52a to K (SM2), would potentially inhibit (or destroy) binding to CD52. In addition, another single charge-reversed variant was produced: K53 to D (SM3). A fourth mutant (DM) carrying two charge differences of K52b to D and K53 to D was also constructed.

52a	52b	53	54	55	
D	K	A	K	G	Y
* D	*	*	*	*	
K	*	*	*	*	
*	*	*	D	*	
* D	*	D	*	*	

"Wild-type" CP-1H

SM1 (K52bD)

SM2 (D52aK)

SM3 (K53D)

DM (K52bD, K53D)

FIGURE 3. H2 loop sequences of CAMPATH-1H and of the four minimal mutants derived from CAMPATH-1H. The six-residue H2 loop sequence of wild-type CAMPATH-1H (CP-1H) is shown with Kabat designation (31) above it. Mutations in the H2 loop of SM1, SM2, SM3, and DM are shown; *, identity to wild-type sequence.

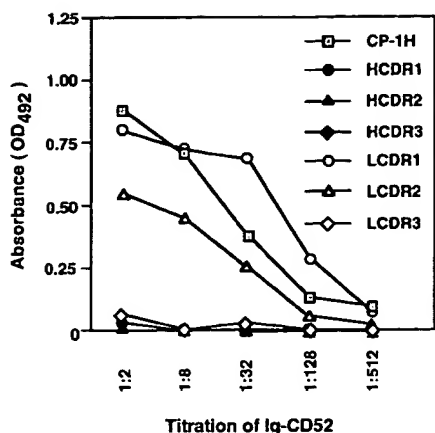


FIGURE 4. Binding of the CAMPATH-1H Fab and mutant Fabs to CD52. Anti-human Fab-coated microtiter plates were used to capture Fab fragments at saturating conditions. The Ig-CD52 fusion protein was then titrated into the wells in doubling dilutions. Bound Ig-CD52 was detected using biotinylated anti-mouse IgG and ExtrAvidin peroxidase.

The ability of the expressed minimal mutants to bind to CD52 was determined, first, by measuring the interaction with Ig-CD52 by ELISA. SM1 bound reasonably well, whereas SM2 bound very weakly, and binding of SM3 or DM was not observed (Fig. 5). Secondly, FACS was used to assay binding of the minimal mutants to lymphocytes isolated from the spleens of CD52 Tg mice. SM1 and SM2 stained cells weakly, whereas binding of SM3 or DM to cells was not detectable (Fig. 6). From these data, SM2, SM3, and DM were selected as potential tolerogens. Based on our previous studies (1, 12), we predicted that the SM3 and DM mutants might

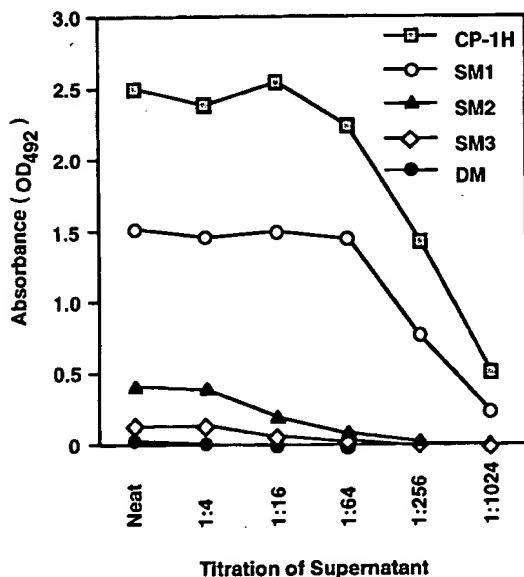


FIGURE 5. Binding of the minimal mutants to Ig-CD52. CAMPATH-1H or minimal mutants from transfection supernatants (all expressing at $\sim 10 \mu\text{g/ml}$) were diluted fourfold in wells of microtiter plates coated with anti-human IgG. Recombinant Ig-CD52 was added at $5 \mu\text{g/ml}$, and bound protein was detected using biotinylated anti-mouse IgG followed by ExtrAvidin peroxidase.

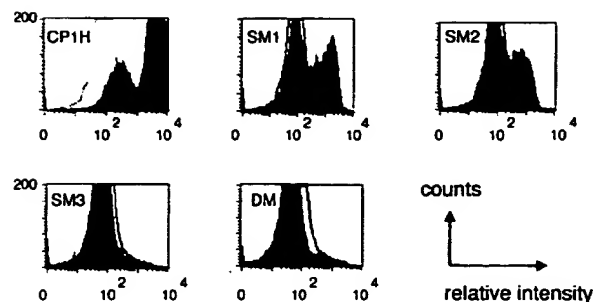


FIGURE 6. Binding of minimal mutants to CD52⁺ lymphocytes. Spleen cells isolated from CD52 Tg mice were incubated for 1 h on ice with purified CAMPATH-1H or minimal mutants ($10 \mu\text{g/ml}$). Bound Ab were detected with biotinylated anti-human IgG and ExtrAvidin-FITC. Profiles of cells incubated with detection reagents alone (anti-human IgG/FITC) are shown as a gray outline superimposed on each sample profile.

be effective tolerogens, as they contain virtually all of the possible Th epitopes of the wild-type mAb, but in a non-cell-binding form. Additionally, we could assess the tolerogenic potential of the SM2 low affinity variant.

Converting CAMPATH-1H to a non-cell-binding form abolishes its immunogenicity

In a preliminary experiment, we determined that CD52 Tg mice made normal antiglobulin responses to foreign Ab. Mice Tg for CD52, or their non-Tg littermates, were given a single i.p. injection of CAMPATH-1H on day 0. Doses of 1, 10, 50, 100, and 250 μg per mouse were given (four mice per group). Twelve days following CAMPATH-1H administration, antiglobulin responses were determined by ELISA. Whereas non-Tg mice were unresponsive to CAMPATH-1H, CD52 Tg mice treated with doses between 1 and 50 μg produced a vigorous antiglobulin response in 10 of 12 mice (titers $> 1/1250$). At these doses of CAMPATH-1H, T cell depletion is limited, but at higher doses, CAMPATH-1H depletes most of the peripheral T cells and has therefore proven immunosuppressive for the antiglobulin response (data not shown).

Subsequently, we measured the immunogenicity of the minimal mutants in CD52 Tg mice. One milligram of monomeric mAb (CAMPATH-1H, SM2, DM, or an irrelevant human IgG1 (anti-human CD4(39)) per mouse was given i.p. on day 0 and again on day 7. Mice were bled 2 weeks following the second dose. ELISA was used to detect antiglobulins against the injected Abs (Fig. 7A). Of the mice receiving SM2, six of eight had titers $\leq 1/20$, with two mice responding, albeit weakly (1/30 and 1/160). All eight DM-treated mice had titers of $\leq 1/20$. In comparison, seven of eight Tg mice treated with monomeric CAMPATH-1H had titers ranging from 1/500 to 1/2560, with one mouse responding weakly (1/125). The non-cell-binding, isotype-matched control Ab (anti-human CD4) did not elicit a response, as expected.

In a second experiment, 12 CD52 Tg mice received an i.p. injection of 1.0 mg of monomeric SM3 on day 0 and again on day 3, and mice were bled 2 weeks following the second injection. Antiglobulin responses were determined as before. Monomeric SM3 was relatively nonimmunogenic in CD52 Tg mice. Eight of 12 mice receiving SM3 had titers $< 1/20$, while 4 mice had very low titers of $\leq 1/80$ (Fig. 7B). Additionally, in an ongoing experiment, eight of nine CD52 Tg mice treated with SM3 as before had antiglobulin titers of $\leq 1/20$ 2 weeks following the second injection. One mouse was responding weakly (1/80). Taken together, the SM3- and DM-treated mice show that the immunogenicity of CAMPATH-1H is destroyed when its cell-binding capacity is abolished.

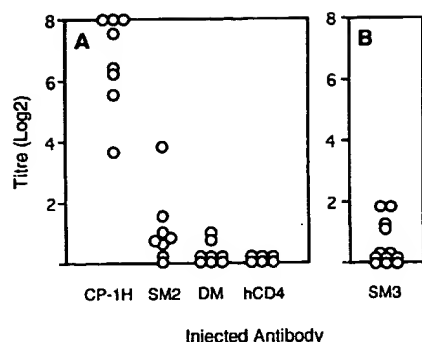


FIGURE 7. Immunogenicity of minimal mutants in vivo. *A*, CD52 Tg mice were treated on day 0 and on day 7 with 1.0 mg of monomeric CAMPATH-1H (CP-1H), SM2, DM, or anti-human CD4 (hCD4). Two weeks later, their sera were screened for antiglobulin responses against these proteins. Sera were serially diluted twofold from a starting dilution of 1/20 into wells of microtiter plates coated with CAMPATH-1H, SM2, DM, or anti-human CD4. Bound antiglobulins were detected with anti-mouse IgG-HRPO. Titers were calculated by comparison to a standard curve (anti-HGG serum). *B*, CD52 Tg mice were treated on day 0 and on day 3 with 1.0 mg of monomeric SM3. Fourteen days later, their sera were screened as before for antiglobulin responses against SM3 using microtiter plates coated with SM3.

Deaggregated non-cell-binding mutants induce tolerance to wild-type CAMPATH-1H

Two experimental designs were used to investigate whether monomeric non-cell-binding minimal mutants could tolerize mice to subsequent challenge with wild-type CAMPATH-1H. In the first protocol (Fig. 8*A*), CD52 Tg mice were injected i.p. with two doses of 1.0 mg of monomeric CAMPATH-1H, SM2, DM, or anti-human CD4, as indicated. Two weeks later, all mice were challenged with three rounds of single-dose (5 μ g) CAMPATH-1H at 10-day intervals. After each challenge, CAMPATH-1H titers were determined. Following the first challenge, mice pretreated with SM2 or DM had consistently very low titers in comparison with CD52 Tg mice that had not been pretreated with these mutants or in comparison with those pretreated with CAMPATH-1H (Fig. 8*B*). Mice pretreated with anti-hCD4 showed an intermediate response. The responses of the DM-pretreated mice remained low throughout successive challenges, although two mice in the DM-treated group became sensitized to CAMPATH-1H following rechallenge (Fig. 8*C*). Tolerance was incomplete in mice pretreated with the weakly binding SM2 mutant, as these mice did eventually make responses to CAMPATH-1H.

In the second protocol, 12 CD52 Tg mice were given an i.p. injection of 1.0 mg of monomeric SM3 on day 0 and again on day 3. Ten CD52 Tg mice were left untreated. Subsequently, all mice received two courses of CAMPATH-1H challenge (5 μ g on each of 5 successive days) 4 weeks apart, as indicated (Fig. 9*A*). Antiglobulin titers to CAMPATH-1H were determined following each challenge course. Ten of 12 mice pretreated with SM3 made no response to CAMPATH-1H after the first challenge course, whereas all non-pretreated mice made strong CAMPATH-1H responses (titers of $>1/2500$) (Fig. 9*B*). After the second challenge course, the effect of SM3 pretreatment was even more dramatic (Fig. 9*C*). The titers of the two responding mice in the SM3-treated group remained low, whereas the mice without SM3 pretreatment responded with titers of $\sim 1/20,000$. This represents a 2000-fold difference between the average response of the SM3-treated and untreated mice (\log_2 titers of 0.5 ± 1.0 vs 11.1 ± 0.7).

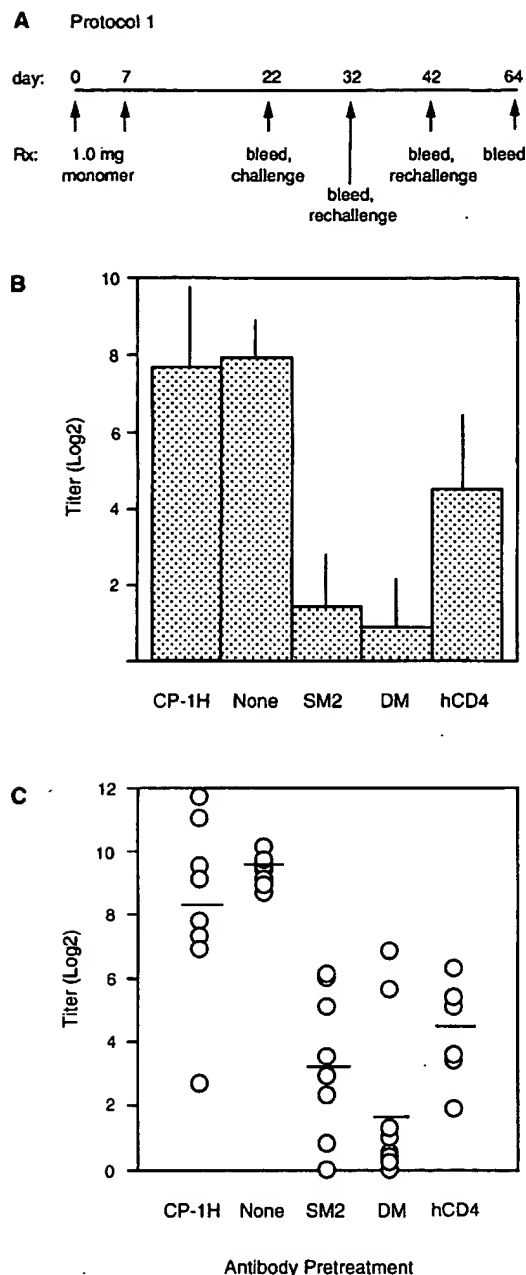


FIGURE 8. CAMPATH-1H antiglobulin responses in CD52 Tg mice pretreated with SM2 or DM. *A*, Tolerizing protocol: CD52 Tg mice were pretreated with two doses of monomeric CAMPATH-1H (CP-1H), SM2, DM, or anti-human CD4 (hCD4), or were left untreated. Two weeks later, all mice received three challenges of single-dose CAMPATH-1H (5 μ g) at 10-day intervals. *B*, Antiglobulin responses to CAMPATH-1H following the first challenge. Sera were serially diluted twofold into microtiter plates coated with CAMPATH-1H. Bound antiglobulins were detected by ELISA using anti-mouse Ig-HRPO. Titers were determined by comparison to a mouse anti-HGG serum standard. *C*, Antiglobulin responses to CAMPATH-1H following the third challenge were determined as before. The average response of each group is indicated.

Antiglobulin responses of mice pretreated with DM are directed to the H2 loop

Terminal-bleed sera from all CD52 Tg mice pretreated with monomeric SM2, SM3, DM, or anti-human CD4 and challenged with CAMPATH-1H were tested for reactivity against both immunizing

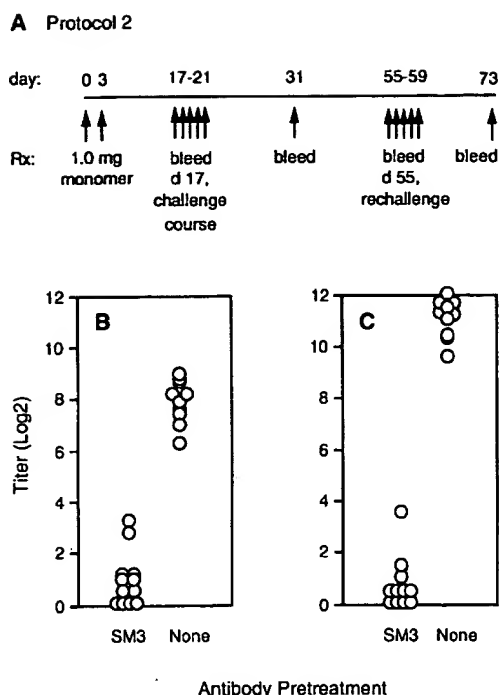


FIGURE 9. CAMPATH-1H antiglobulin responses in CD52 Tg mice pretreated with SM3. *A*, Tolerizing protocol: CD52 Tg mice were pretreated with two doses of monomeric SM3 as indicated or were left untreated. Two weeks later, all mice received two challenge courses of CAMPATH-1H (5 μ g per day over 5 days) 4 weeks apart. *B*, Antiglobulin responses of individual mice to CAMPATH-1H following the first challenge course were determined by ELISA. Sera were serially diluted two-fold into wells of microtiter plates coated with CAMPATH-1H. Antiglobulins were detected with anti-mouse Ig-HRPO, and titers were determined by comparison to a mouse anti-HGG serum standard. *C*, Antiglobulin responses of individual mice to CAMPATH-1H following the second challenge course were determined as before.

Ab and CAMPATH-1H. Mice pretreated with SM2 produced antiglobulin responses to SM2 over time, with titers ranging from 1/40 to 1/1280 (Fig. 10*A*), although the mutant binds cells only weakly. Tolerance to CAMPATH-1H was achieved in only two of

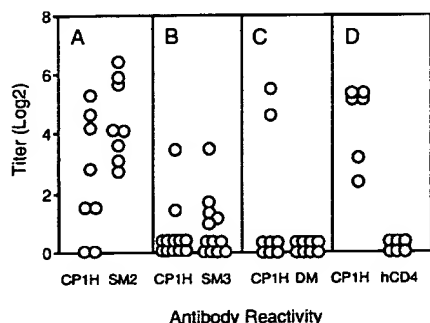


FIGURE 10. Inducing tolerance to the H2 loop of CAMPATH-1H. Terminal bleed sera from mice pretreated with SM2, SM3, DM, or hCD4, obtained following repeated CAMPATH-1H challenge, were tested for reactivity against these Ab and against CAMPATH-1H. Microtiter plates were coated with the appropriate mutant or with CAMPATH-1H, and binding of antiglobulins was detected by ELISA as before. Individual responses of CD52 Tg mice pretreated with SM2 (*A*), with SM3 (*B*), with DM (*C*), or with hCD4 (*D*) are shown.

eight mice in this group. Most mice pretreated with SM3 became tolerant and remained unresponsive to SM3 for the duration of the experiment (Fig. 10*B*). Seven of 12 mice had titers <1/20, and 4 of 12 mice had very low titers of 1/20 to 1/40. Two of the low responders also responded weakly to CAMPATH-1H (titers of ~1/40 and ~1/160). The one mouse that responded appreciably to SM3 was still unresponsive to CAMPATH-1H. In the DM-pretreated group (Fig. 10*C*), six of eight mice were tolerant to CAMPATH-1H upon repeated challenge, and again, the two responders remained unresponsive to the DM mutant. These results show that the two responders were not fully tolerant to CAMPATH-1H V regions, as both mice produced anti-idiotypic Abs that recognized the original H2 sequence, but not the mutated version carrying Asp residues at positions 52b and 53. In contrast, none of the mice pretreated with anti-human CD4 were tolerant to CAMPATH-1H V-region determinants (Fig. 10*D*), although none reacted to hCD4 Ab carrying the same C-region residues as CAMPATH-1H (C-region tolerance).

Discussion

Monomeric (deaggregated) foreign serum proteins given without adjuvants can induce tolerance in adult immunocompetent animals (13–16, 40–43). Th cells can be tolerized at low Ag doses, whereas combined T and B cell tolerance requires high doses. In contrast, Abs that bind to hemopoietic cells have enhanced immunogenicity, even at low doses (1). So powerful is the cell-binding effect, that antiglobulin responses arise even to Abs known to be immunosuppressive (7–10, 44). We have shown here that immunogenic cell-binding Abs can be rendered tolerogenic by mutating essential residues that destroy Ag binding. This suggests the prospect of pretolerization to enable repeated use of cell-binding Abs in individual patients.

If we could be certain that all mAb Th epitopes would be presented efficiently for tolerance and that all Th cells would be tolerized, then low zone tolerance should be sufficient. Conservation of structure in the non-cell-binding mutants would be less important, because anti-idiotypic B cells would not be activated in the absence of T cell help. However, we would have a greater chance to achieve tolerance to the wild-type mAb if anti-idiotypic B cells were tolerized directly, in addition to T cells (high-zone tolerance). A subtle mutagenesis approach is needed if structural B cell epitopes are to be preserved in a non-cell-binding mutant. If crystal structure is available (preferably cocrystallized with Ag), then it is relatively straightforward to choose a key mutation that would maintain structure of the Ab while disrupting Ag binding. In many cases, a molecular model could provide the necessary information. Comparative model building of Ab is now widely used, and the structural databases and programs available allow the generation of highly reliable models (45). For example, solved crystal structure for CAMPATH-1H (46) follows the predicted canonical model (38). Sequential alanine or serine scanning through CDR loops, while avoiding key residues that define canonical structures and those that are close to framework regions (38), could augment information provided by molecular modeling in the absence of crystal structure. Scanning could also be used in lieu of the model itself. As certain residues, especially Tyr, Trp or Ser, or charged residues (47, 48), are often involved in contacts with Ag, sequential mutation of those residing in Ag-binding CDRs to alanine or serine may identify key interactions.

We generated a rapid method (CDR swapping) for identification of CDRs important for CD52 binding. These results allowed us to focus initially on H1, H2, H3, and L3 as regions for

mutagenesis. A combination of molecular modeling, and recently obtained data from the CAMPATH-1H crystal structure (46), narrowed our choice to the H2 loop. We then concentrated on the charged residues in H2 and reversed these charges for the most dramatic effect on Ag binding. It is impressive that changing a single charged residue (Lys⁵³ to Asp) in the H2 loop of CAMPATH-1H abolished Ag binding. The crystal structure of CAMPATH-1H shows a large positively charged surface formed by the solvent exposed side chains of K52b and K53 that could interact with negatively charged phosphate groups in the GPI anchor (46). Our ELISA results with the SM1 (K52bD) and SM3 (K53D) mutants (Fig. 5) suggest that of the two residues, Lys53 is critical for binding CD52.

When SM3 and DM (also carrying the K53D mutation, in addition to K52bD) were injected into CD52 Tg mice, the mutants did not elicit antiglobulin responses. These *in vivo* results support ELISA and FACS data demonstrating their conversions to non-cell-binding forms of CAMPATH-1H. Comparison of the response elicited by wild-type CAMPATH-1H to the lack of response against SM3 and DM shows definitively the strength of the cell-binding effect (1). These data emphasize the need for further strategies to avoid Ab immunogenicity, in addition to the well-established humanization approach (25), as we know that even humanized cell-binding Ab can elicit anti-idiotypic Ab in some patients (7–10).

The use of Tg mice expressing human CD52 allowed us to test both immunogenicity as well as the tolerogenicity of the minimal mutants *in vivo*. CD52 is expressed on lymphocytes and monocytes in humans (2), and CAMPATH-1H mAb effectively targets these cells for destruction. In CD52 Tg mice, the CD2 promoter targets high level expression of CD52 to mouse T cells, which would have been the cells most at risk of depletion following CAMPATH-1H injection. The experimental design was based on the requirement that we should avoid too high a challenge dose of wild-type CAMPATH-1H *in vivo*, for fear that this would deplete T cells, and so leave the mice immunocompromised. We therefore determined the optimal challenge of CAMPATH-1H by a dose-ranging study and chose the highest dose that could be given without significant T cell depletion. Indeed, we observed that a supraoptimal dose of 100 μ g per mouse or greater resulted in antiglobulin titers that were correspondingly low.

In subsequent tolerance experiments, the selected challenge dose was sufficient to elicit strong antiglobulin responses in mice that were not pretolerized or in mice receiving monomeric wild-type CAMPATH-1H Ab. These responses increased upon subsequent challenge, as did the responses of mice pretreated with the low affinity binding-variant, SM2. In contrast, later bleeds of mice pretreated with the nonbinding SM3 or DM mutants demonstrated that these mice did not regain their response to wild-type CAMPATH-1H.

We noted tolerance to both V and C regions (pretreatment with the non-cell-binding CAMPATH-1H derivatives SM3 or DM) or tolerance to C region only (nonbinding isotype-matched control mAb). CD52 Tg mice that were pretreated with large doses of monomeric nonbinding SM3 or DM mutants were tolerant to subsequent challenge with wild-type CAMPATH-1H. In contrast, all mice pretreated with the irrelevant anti-human CD4 mAb responded on challenge with CAMPATH-1H, these responses being wholly directed to the V region of CAMPATH-1H, but not to the C regions. Therefore, the partial tolerogenic effect observed with anti-human CD4 mAb in CD52 Tg mice must be attributed to C-region tolerance. The SM2 mutant that binds weakly to cells did not tolerize, and indeed responses were generated to both V and C

region determinants. Although the SM3 or DM mutants induced tolerance in most mice, the few animals with detectable Ab titers in the DM-treated group made responses directed just to CAMPATH-1H and not to the mutant forms. By inference, these responses are against heavy chain H2, since the mutants differ there from CAMPATH-1H by only one or two residues.

This work has clear implications for human therapy. We can see applications of the concept to a number of diseases. Minimal mutants could be given as an "one-off" treatment before mAb therapy for transplant rejection or autoimmunity. If the patient relapsed, one could readminister non-cell-binding mAb before each course of therapeutic mAb. We expect this approach to extend to cancer therapy, although immunosuppressive diseases such as chronic lymphocytic leukemia may not even require pretolerization. Because we cannot yet predict which patients will produce an anti-idiotypic response, it would seem appropriate to administer the tolerogenic non-cell-binding mutant to all who might eventually merit repeat treatment with the therapeutic cell-binding form. In the future, it may be possible to determine a genetic link between those that respond and particular DR/DQ alleles that react with particular Th peptide(s), so that only the at-risk individuals would need prophylaxis.

These findings should encourage additional avenues to reducing Ab immunogenicity. If the Ab-derived Th epitopes could be identified, we could target Ag presentation and processing. The binding of Th peptide(s) to MHC class II molecules could be altered by changing anchor residues (49). Alternatively, it may be possible to alter processing of the peptides. EBNA-1 (EBV nuclear Ag) Gly-Ala repeats generate an inhibitory signal that interferes with Ag processing during MHC class I-restricted presentation (50, 51). Altered peptide ligands could be used to destroy (or significantly reduce) the TCR/peptide interaction through mutating Ab CDR(s) at residues that did not affect binding, but did diminish affinity for the TCR (52, 53) such that the T cells are rendered partially anergic and unable to respond to subsequent stimulation (54, 55).

Despite significant advances (56–58), reliable identification of all Th epitopes for any given protein sequence is not yet possible. For this reason, we favor the tolerance strategy, which requires no prior knowledge of the Th epitopes. Although we do not understand the mechanism employed by the non-cell-binding mutants to induce tolerance to the cell-binding form, it is likely to be similar to tolerance induced by deaggregated HGG. Peripheral tolerance in CD4⁺ T cells induced by deaggregated HGG has been shown to be independent of FcRI and FcRII signaling (59), and B cells are not required (60). The key issue may be that postulated by Chiller et al. (15, 16) in the 1970s, that serum persistence plays a role in the induction of tolerance to HGG. Additionally we know that Ag persistence is necessary to reinforce (maintain) tolerance (61). It follows that a minimal mutant Ab would be a better tolerogen than overlapping peptides due to the long half-life of IgG *in vivo* (62, 63).

Our results show that it is possible to abolish the antiglobulin response to a cell-binding Ab by first tolerizing with a non-cell-binding variant that possesses essentially all the Th and most of the B cell epitopes. We also present a rapid method (CDR swapping) to determine which CDRs to target for creation of a tolerogenic mutant. In many cases, an SM may not be related to a region that can constitute a T cell epitope. If so, then full tolerogenicity should be possible. One caveat is that even an SM in a nonbinding mutant may destroy a Th epitope so that it is not presented for tolerance. If T cell tolerance is incomplete,

then the anti-idiotypic B cell might attain the necessary response against the wild-type epitopes. One way to avoid incomplete tolerance might be to produce two tolerogenic variants for each therapeutic Ab, each containing a key mutation(s) localized to different Ag-binding CDR. A further approach in the case of the CAMPATH-1H Ab might be to remove some of the more "immunogenic" surface charges that might be preferred targets for anti-idiotypic Ab. When considering the optimal design of a tolerogenic derivative of a therapeutic Ab, it may be necessary to incorporate the following attributes: 1) non-cell binding with minimal mutations to conserve both T and B cell epitopes and 2) an isotype to encourage serum persistence.

Acknowledgments

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References

- Benjamin, R. J., S. P. Cobbold, M. R. Clark, and H. Waldmann. 1986. Tolerance to rat monoclonal antibodies: implications for serotherapy. *J. Exp. Med.* 163: 1539.
- Hale, G., M.-Q. Xia, H. P. Tighe, M. J. S. Dyer and H. Waldmann. 1990. The CAMPATH-1 antigen (CDw52). *Tissue Antigens* 35:118.
- Xia, M.-Q., M. Tone, L. Packman, G. Hale, and H. Waldmann. 1991. Characterization of the CAMPATH-1 (CDw52) antigen: biochemical analysis and cDNA cloning reveal an unusually small peptide backbone. *Eur. J. Immunol.* 21:1677.
- Treumann, A., M. R. Lively, P. Schneider, and M. A. J. Ferguson. 1995. Primary structure of CD52. *J. Biol. Chem.* 270:6088.
- Xia, M.-Q., G. Hale, M. R. Lively, M. A. J. Ferguson, D. Campbell, L. Packman, and H. Waldmann. 1993. Structure of the CAMPATH-1 antigen, a glycosylphosphatidylinositol-anchored glycoprotein which is an exceptionally good target for complement lysis. *Biochem. J.* 293:633.
- Xia, M.-Q., G. Hale, and H. Waldmann. 1993. Efficient complement-mediated lysis of cells containing the CAMPATH-1 (CDw52) antigen. *Mol. Immunol.* 30: 1089.
- Isaacs, J. D., R. A. Watts, B. L. Hazleman, G. Hale, M. T. Keogan, S. P. Cobbold, and H. Waldmann. 1992. Humanised monoclonal antibody therapy for rheumatoid arthritis. *Lancet* 340:748.
- Lockwood, C. M., S. Thiru, J. D. Isaacs, G. Hale, and H. Waldmann. 1993. Long-term remission of intractable systemic vasculitis with monoclonal antibody therapy. *Lancet* 341:1620.
- Isaacs, J. D., V. K. Manna, N. Rapson, K. J. Bulpitt, B. L. Hazleman, E. L. Matteson, E. W. St.-Clair, T. J. Schnitzer, and J. M. Johnston. 1996. CAMPATH-1H in rheumatoid arthritis: an intravenous dose-ranging study. *Br. J. Rheumatol.* 35:231.
- Weinblatt, M. E., P. J. Maddison, K. J. Bulpitt, B. L. Hazleman, M. B. Urowitz, R. D. Sturrock, J. S. Coblyn, A. L. Maier, W. R. Spreen, and V. K. Manna. 1995. CAMPATH-1H, a humanized monoclonal antibody, in refractory rheumatoid arthritis: an intravenous dose-escalation study. *Arthritis Rheum.* 38:1589.
- Isaacs, J. D. 1990. The antiglobulin response to therapeutic antibodies. *Semin. Immunol.* 2:449.
- Isaacs, J. D., and H. Waldmann. 1994. Helplessness as a strategy for avoiding anti-globulin responses to therapeutic monoclonal antibodies. *Ther. Immunol.* 1:303.
- Weigle, W. O. 1973. Immunological unresponsiveness. *Adv. Immunol.* 16:61.
- Weigle, W. O. 1980. Analysis of autoimmunity through experimental models of thyroiditis and allergic encephalomyelitis. *Adv. Immunol.* 30:159.
- Chiller, J. M., G. S. Habicht, and W. O. Weigle. 1970. Cellular sites of immunologic unresponsiveness. *Proc. Natl. Acad. Sci. USA* 65:551.
- Chiller, J. M., G. S. Habicht, and W. O. Weigle. 1971. Kinetic differences in unresponsiveness of thymus and bone marrow cells. *Science* 171:813.
- Jungmans, R. P., and C. L. Anderson. 1996. The protection receptor for IgG catabolism is the β_2 -microglobulin-containing neonatal intestinal transport receptor. *Proc. Natl. Acad. Sci. USA* 93:5512.
- Ghetie, V., J. G. Hubbard, J.-K. Kim, M.-F. Tsen, Y. Lee, and E. S. Ward. 1996. Abnormally short serum half-lives of IgG in β_2 -microglobulin-deficient mice. *Eur. J. Immunol.* 26:690.
- Vaughan, T. J., J. K. Osbourn, and P. R. Tempest. 1998. Human antibodies by design. *Nat. Biotechnol.* 16:535.
- Bruggemann, M., and M. J. Taussig. 1997. Production of human antibody repertoires in transgenic mice. *Curr. Opin. Biotechnol.* 8:455.
- Cox, J. P. L., I. M. Tomlinson, and G. Winter. 1994. A directory of human germ-line V κ genes reveals a strong bias in their usage. *Eur. J. Immunol.* 24: 827.
- Cook, G. P., and I. M. Tomlinson. 1995. The human immunoglobulin VH repertoire. *Immunol. Today* 16:237.
- Williams, S. C., J.-P. Fripiat, I. M. Tomlinson, O. Ignatovich, M.-P. Lefranc, and G. Winter. 1996. Sequence and evolution of the human germline V λ repertoire. *J. Mol. Biol.* 264:220.
- Clackson, T., H. R. Hoogenboom, A. D. Griffiths, and G. Winter. 1991. Making antibody fragments using phage display libraries. *Nature* 352:624.
- Riechmann, L., M. Clark, H. Waldmann, and G. Winter. 1988. Reshaping human antibodies for therapy. *Nature* 332:323.
- Hoogenboom, H. R., A. D. Griffiths, K. S. Johnson, D. J. Chiswell, P. Hudson, and G. Winter. 1991. Multi-subunit proteins on the surface of filamentous phage: methodologies for displaying antibody (Fab) heavy and light chains. *Nucleic Acids Res.* 19:4133.
- Saul, F. A., L. M. Amzel, and R. J. Poljak. 1978. Preliminary refinement and structural analysis of the Fab fragment from human immunoglobulin NEW at 2.0 Å resolution. *J. Biol. Chem.* 253:585.
- Epp, O., P. Colman, H. Fehlimmer, W. Bode, M. Schiffer, R. Huber, and W. Palm. 1974. Crystal and molecular structure of a dimer composed of the variable portions of the Bence-Jones protein REI. *Eur. J. Biochem.* 45:513.
- Horton, R. M., H. D. Hunt, S. N. Ho, J. K. Pullen, and L. R. Pease. 1989. Engineering hybrid genes without the use of restriction enzymes: gene splicing by overlap extension. *Gene* 77:61.
- Gunning, P., J. Leavitt, C. Muscat, S.-Y. Ng, and L. Kedes. 1987. A human β -actin expression vector system directs high-level accumulation of antisense transcripts. *Proc. Natl. Acad. Sci. USA* 84:4831.
- Kabat, E. A., T. T. Wu, H. M. Perry, K. S. Gottesman, and C. Foeller. 1991. *Sequences of Proteins of Immunological Interest*, 5th Ed. U.S. Department of Health and Human Services, Washington, DC.
- Page, M. J., and M. A. Sydenham. 1991. High level expression of the humanized monoclonal antibody Campath-1H in Chinese hamster ovary cells. *Biotechnology* 9:64.
- Lang, G., D. Wotton, M. J. Owen, W. A. Sewell, M. H. Brown, D. Y. Mason, M. J. Crumpton, and D. Kioussis. 1988. The structure of the human CD2 gene and its expression in transgenic mice. *EMBO J.* 7:1675.
- Greaves, D. R., F. D. Wilson, C. Lang, and D. Kioussis. 1989. Human CD2 3'-flanking sequences confer high-level, T-cell specific, position-independent gene expression in transgenic mice. *Cell* 56:979.
- Searle, S. J., J. T. Pedersen, A. H. Henry, D. M. Webster, and A. R. Rees. 1995. Antibody structure and function. In *Antibody Engineering*, 2nd Ed. C. A. K. Borrebaeck, ed. Oxford University Press, Oxford, UK, p. 3.
- McCafferty, J., A. D. Griffiths, G. Winter, and D. J. Chiswell. 1990. Phage antibodies: filamentous phage displaying antibody variable domains. *Nature* 348:552.
- Chang, C.N., N. F. Landolfi, and C. Queen. 1991. Expression of antibody Fab domains on bacteriophage surfaces: potential use for antibody selection. *J. Immunol.* 147:3610.
- Chothia, C., and A. M. Lesk. 1987. Canonical structures for the hypervariable regions of immunoglobulins. *J. Mol. Biol.* 196:901.
- Gorman, S. D., M. R. Clark, E. G. Routledge, S. P. Cobbold, and H. Waldmann. 1991. Reshaping a therapeutic CD4 antibody. *Proc. Natl. Acad. Sci. USA* 88: 4181.
- Dresser, D. W. 1962. Specific inhibition of antibody production. II. Paralysis induced in adult mice by small quantities of protein antigen. *Immunology* 5:378.
- Mitchison, N. A. 1964. Induction of immunological paralysis in two zones of dosage. *Proc. R. Soc. Lond. Ser. B Biol. Sci.* 161:275.
- Dietrich, F. M., and W. O. Weigle. 1964. Immunological unresponsiveness to heterologous serum proteins induced in adult mice and transfer of the unresponsive state. *J. Immunol.* 92:167-172.
- Gahring, L. C., and W. O. Weigle. 1989. The induction of peripheral T cell unresponsiveness in adult mice by monomeric human γ -globulin. *J. Immunol.* 143:2094.
- Jonker, M., and F. J. Nooij. 1987. The internal image-like anti-idiotypic response to a CD3-specific monoclonal antibody in primates is dependent on the T-cell-binding properties of the injected antibody. *Eur. J. Immunol.* 17:1519.
- Bajorath, J., and J. Novotny. 1995. Model building of antibody combining sites. *Ther. Immunol.* 2:95.
- Cheetham, G. M. T., Hale, G., Waldmann, H., and Bloomer, A. C. 1998. Crystal structures of a rat anti-CD52 (CAMPATH-1) therapeutic antibody Fab and its humanized counterpart. *J. Mol. Biol.* 284:85.
- Mian, I. S., Bradwell, A. R., and Olson, A. R. 1991. Structure, function and properties of antibody binding sites. *J. Mol. Biol.* 217:133.
- Braden, B. C., and R. J. Poljak. 1995. Structural features of the reactions between antibodies and protein antigens. *FASEB J.* 9:9.
- Brown, J. H., T. S. Jardetzky, J. C. Gorga, L. J. Stern, R. G. Urban, J. L. Strominger, and D. C. Wiley. 1993. Three-dimensional structure of the human class II histocompatibility antigen HLA-DR1. *Nature* 364:33.
- Levitskaya, J., M. Coram, V. Levitsky, S. Imreh, P. M. Steigerwald-Mullen, G. Klein, M. G. Kurilla, and M. G. Masucci. 1995. Inhibition of antigen processing by the internal repeat region of the Epstein-Barr virus nuclear antigen-1. *Nature* 375:685.
- Sharipo, A., M. Imreh, A. Leonchiks, S. Imreh, and M. G. Masucci. 1998. A minimal glycine-alanine repeat prevents the interaction of ubiquitinated I κ B α with the proteasome: a new mechanism for selective inhibition of proteolysis. *Nat. Med.* 4:939.

52. Fairchild, P. J. 1997. Altered peptide ligands: prospects for immune intervention in autoimmune disease. *Eur. J. Immunogenet.* 24:155.
53. Sloan-Lancaster, J., and P. M. Allen. 1996. Altered peptide ligand-induced partial T cell activation: molecular mechanisms and role in T cell biology. *Annu. Rev. Immunol.* 14:1.
54. Sloan-Lancaster, J., B. D. Evavold, and P. M. Allen. 1993. Induction of T-cell anergy by altered T-cell-receptor ligand on live antigen-presenting cells. *Nature* 363:156.
55. Sloan-Lancaster, J., B. D. Evavold, and P. M. Allen. 1994. Th2 cell clonal anergy as a consequence of partial activation. *J. Exp. Med.* 180:1195.
56. Hammer, J., P. Valsasini, K. Tolba, D. Bolin, J. Higelin, B. Takacs, and F. Sinigaglia. 1993. Promiscuous and allele-specific anchors in HLA-DR-binding peptides. *Cell* 74:197.
57. Hammer, J. 1995. New methods to predict MHC-binding sequences within protein antigens. *Curr. Opin. Immunol.* 7:263.
58. Sette, A., S. Buus, E. Appella, J. A. Smith, R. Chesnut, C. Miles, S. M. Colon, and H. M. Grey. 1989. Prediction of major histocompatibility complex binding regions of protein antigens by sequence pattern analysis. *Proc. Natl. Acad. Sci. USA* 86:3296.
59. Whitmer, K. J., Romball, C. G., and Weigle, W. O. 1997. Induction of tolerance to human γ -globulin in FcR γ - and Fc γ RII-deficient mice. *J. Immunol.* 159:644.
60. Phillips, J. A., Romball, C. G., Hobbs, M. V., Ernst, D. N., Shultz, L., and Weigle, W. O. 1996. CD4+ T cell activation and tolerance induction in B cell knockout mice. *J. Exp. Med.* 183:1339.
61. Scully, R., S. Qin, S. Cobbold, and H. Waldmann. 1994. Mechanisms in CD4 antibody-mediated transplantation tolerance: kinetics of induction, antigen dependency and role of regulatory T cells. *Eur. J. Immunol.* 24:2383.
62. Spiegelberg, H. L., and W. O. Weigle. 1965. The catabolism of homologous and heterologous 7S gamma globulin fragments. *J. Exp. Med.* 121:323.
63. Zuckier, L. S., L. D. Rodriguez, and M. D. Scharff. 1989. Immunologic and pharmacologic concepts of monoclonal antibodies. *Semin. Nucl. Med.* 19:166.